



Short Communication

Effects of feed restriction and forage:concentrate ratio on digestibility, methane emission, and energy utilization by goats

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ABSTRACT - This study was carried out to evaluate how feed restriction and different forage:concentrate ratios affect digestibility, methane emission (using the SF₆ technique), and energy utilization of Anglo-Nubian goats. Fifteen (15) dry and non-pregnant Anglo Nubian goats, averaging 30±2.9 kg body weight, were used. The experiment was divided into two trials, the first of which was designed to study the effects of feed restriction (0% or *ad libitum*; 15% of feed restriction or equivalent to 85% of *ad libitum* intake; and 40% of feed restriction or equivalent to 60% of *ad libitum* intake) and the second, to study the effects of forage:concentrate (F:C) ratios (75:25, 54:46, and 25:75) in the diet. The sulfur hexafluoride (SF₆) tracer gas method was used to collect and estimate methane (CH₄) emissions. Feed restriction level did not affect apparent total tract digestibility of dry matter (DM), organic matter, crude protein, and neutral detergent fiber. Methane emission (g d⁻¹) decreased linearly as intake level decreased. However, energy loss in methane proportional to organic matter intake was similar among levels of feed restriction; consequently, dietary metabolizability did not differ among treatments. Methane gas (g d⁻¹) as a function of F:C ratio revealed a quadratic response, showing the highest values when animals were fed the 46:54 F:C ratio diet (18.2 g d⁻¹), suggesting that the decrease in absolute CH₄ occurred when the level of concentrate inclusion in the diet surpassed approximately 50%. The results presented herein may be relevant for the ongoing and future efforts towards completion of an IPCC inventory regarding the contribution of goats to the greenhouse gas effects on the planet.

Key Words: greenhouse gases, ruminants, SF₆

Introduction

In environments where natural resources are deficient, where food supply is restricted and/or of poor quality, differences in energy requirements and digestive efficiency, based on the efficiency of gross energy use for production, are important criteria in choosing the most appropriate animal to raise (Devendra, 1990). In these harsh environments, goats are an excellent option for animal production because of their greater adaptability and resistance, smaller body size, high digestive efficiency, and ability to reduce their metabolism (Silanikove, 2000). In this sense, feed restriction, both quantitatively and qualitatively, is an everyday situation faced by goats in worldwide production systems, requiring studies that

characterize and explain the responses of animals under these conditions. In addition, ruminants, including goats and sheep, are raised extensively in these deficient regions, which has caused concern about emission of greenhouse gases. The Intergovernmental Panel for Climate Change (IPCC, 2006) assumed methane (CH₄) emission from goats at 5 kg head⁻¹ per year (Tier 1 methodology), which averages 13 g head⁻¹ per day. However, in recent reviews, Hristov et al. (2013) inferred that sheep and goats produce 10 to 16 kg CH₄ head⁻¹ per year (27 to 44 g d⁻¹), depending on the feed strategy adopted. On the other hand, Fernández et al. (2013) implied that the estimate of IPCC could lead to an overestimate of enteric CH₄ for dairy goats fed concentrate diets.

Apart from being a greenhouse gas, methane also represents significant energy loss to the animal. Diet composition and intake are the main factors affecting methane production by ruminants (Archimede et al., 2011; Hristov et al., 2013); therefore, strategies such as offering high levels of grains or offering different quantities of feed have been evaluated in cattle and sheep. On the other hand, as the microbial community composition in the rumen

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can be strongly influenced by differences in diet, species, genotype, and environment, among other factors (Janssen and Kirs, 2008), one might raise the question about different CH₄ emission profiles for goats, when facing similar conditions to those previously imposed on cattle or sheep. Therefore, the aim of this study was to evaluate how feed restriction and different forage:concentrate ratios affect digestibility, methane emission (using the SF₆ technique), and energy utilization of Anglo-Nubian goats.

Material and Methods

This study was conducted in Jaboticabal, São Paulo State, Brazil (21°14'05"S and 48°17'09"W, altitude 595 m). Humane animal care and handling procedures followed the guidelines set by the Committee on Ethical and Animal Welfare (CBEA; Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista - Unesp, Jaboticabal campus). The project was approved by CBEA (case no. 004972-09).

Fifteen dry and non-pregnant adult Anglo Nubian goats, averaging 30±2.93 kg body weight (BW), were used in this study. The experiment was divided into two trials, the first of which was designed to study the effects of feed restriction, and the second, to study the effects of the forage:concentrate (F:C) ratio in the diet on digestion, methane production, and energy balance.

Trial 1 comprised 26 days. Goats were distributed into five groups (blocks), according to BW and body condition score, with three animals per group. Within each group, goats were allocated to one of three treatments: fed *ad libitum* (AL); 15% of feed restriction (equivalent to 85% of *ad libitum* intake); and 40% of feed restriction (equivalent to 60% of *ad libitum* intake). The restricted-intake amounts were determined daily within each group based on the dry matter intake of goats on the *ad libitum* treatment on the previous day. Days 1 to 21 were used to adapt the animal to treatments and metabolic cages. The metabolism assay and measurement of methane were performed from day 22 to 26. All animals received the same diet, which consisted of dehydrated corn (*Zea mays*) plants, cracked corn grain, soybean (*Glycine max*) meal, molasses, soybean oil, limestone, and a mineral supplement at a 46:54 F:C ratio (Table 1). The dehydrated corn plants consisted of whole corn plants (60 to 70% moisture) chopped when the kernel milk line was approximately two-thirds of the way down the kernel. The chopped material was air-dried for approximately 72 h or until it reached 8 to 10% moisture, and then ground to pass through a 4-mm screen (Wiley-type mill). The experimental diet was formulated to meet

the maintenance requirements (NRC, 2007). Feed was weighed and offered twice daily, at 08.00 and 16.00 h.

Goats were housed in metabolic cages to allow simultaneous measurements of feed intake, total fecal and urinary output, and methane production during five days. Each day, a sub-sample of the feed offered was collected; these were combined and stored at -20 °C. The collected urine was acidified daily with 20 mL of 6 M HCl. Daily refusals of feed, and output of feces and urine were also measured, sub-sampled (10%), and stored at -20 °C. Composites of feed, feed refusals, and feces were dried at 60 to 65 °C for 72 h and ground through a 1 mm screen using a Wiley mill for further analysis. Composite samples of urine were passed through a sieve to remove the large particles, and a subsample was taken for further analysis.

The sulfur hexafluoride (SF₆) tracer gas method was used to collect and estimate methane emissions (Johnson et al., 1994; Boadi et al., 2002), with adjustments to better fit for goats, including the dimensions of PVC canisters. Methane was sampled daily from each goat for five days. Firstly, stainless steel permeation tubes (12.5 mm × 40 mm) were charged with 500 mg of SF₆ at liquid nitrogen temperatures, and incubated at 39 °C. Predetermined release rates of SF₆ were achieved by measuring the weight loss of tubes for 8 wk prior to rumen insertion. Sulfur hexafluoride release rates ranged from 500 to 800 ng/min, which were assumed constant or linear during the experimental period. These stainless steel permeation tubes were placed in the rumen per gavage of all goats a week prior to the start of the experiment, allowing enough time for the tracer gas to equilibrate in the rumen. During this period, animals were

Table 1 - Ingredients and chemical composition of diets

Item	Forage:concentrate ratio		
	75:25	46:54	25:75
Ingredient (g kg ⁻¹ DM)			
Dehydrated corn plant	750	465	250
Cracked corn	6	302	515
Soybean meal	189	190	192
Soybean oil	15	11	11
Limestone	9	7	7
Mineral salt ¹	22	16	16
Ammonium chloride	9	9	9
Chemical composition			
DM (g kg ⁻¹ as fed)	914	909	906
Ash (g kg ⁻¹ DM)	72	63	62
CP (g kg ⁻¹ DM)	178	195	207
EE (g kg ⁻¹ DM)	21	47	47
NDF (g kg ⁻¹ DM)	455	342	255
GE (MJ kg ⁻¹ DM)	16.8	16.9	16.9

DM - dry matter; CP - crude protein; EE - ether extract; GE - gross energy; NDF - neutral detergent fiber.

¹ Composition of mineral supplement, per kg: 65 g P; 180 g Ca; 70 g Na; 100 g Cl; 80 g Mg; 38 g S; 4,000 mg Zn; 100 g Co; 1,500 mg Mn; 1,100 mg Fe; 150 mg I; 25 mg Se.

trained to wear the gas collection apparatus. Expired gases were drawn into pre-evacuated (-12.00 to -12.60 psi) PVC canisters (100 mm diameter, 280 mm length) through a 900 mm length of capillary tubing with an inline filter and flexible nose piece. Collection apparatuses were hung on the east and west sides of the facility, protected from the rain and wind ventilation, each day, to collect background air samples, which were used to correct expired gas concentrations. Collected gas canisters were checked for pressure to identify blocked or leaking capillary systems. Canisters were then pressurized to 1.4 psi with pure N₂ to prevent sample contamination prior to analyses, and to allow injection of gas samples into the sample loop of a gas chromatograph. A gas chromatograph (Agilent® model 6890; Agilent Technologies, Santa Clara CA, USA) fitted with an electron capture detector was used to determine SF₆, and a flame ionization detector was used to determine methane concentration in collected samples. Daily methane production was calculated as follows (Johnson et al., 1994):

$$\text{CH}_4 (\text{L min}^{-1}) = \text{SF}_6 (\text{L min}^{-1}) \times [\text{CH}_4]/[\text{SF}_6]$$

in which SF₆ is the predetermined release rate from the permeation tube; [CH₄] and [SF₆] are the concentrations of methane and SF₆ in samples after background concentrations have been deducted.

Gross energy (GE) intake was determined using heat of combustion of feed and feed refusal. Digestible energy intake was calculated as the difference between GE intake and fecal energy output. Metabolizable energy (ME) intake was determined as the difference between digestible energy intake, and urinary and methane energy output. Methane gas volume was converted to energy and mass values using the conversion factors 39.54 kJ L⁻¹ and 0.716 g L⁻¹, respectively (Brouwer, 1965).

Samples of feed, orts, and feces were analyzed for analytical DM at 105 °C for 24 h, ash content (complete combustion in a muffle furnace at 600 °C for 6 h; AOAC, 1990; method number 924.05), total N (Leco FP-2000 Nitrogen Analyzer, Leco Instruments Inc., St. Joseph, MI; Etheridge et al., 1998), fat (based on weight loss of the dry sample upon extraction with petroleum ether in a Soxhlet extraction apparatus for 6 h; AOAC, 1990; method number 930.15), neutral detergent fiber (NDF) according to Van Soest et al. (1991), adapted for the Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY), acid detergent fiber (ADF; Goering and Van Soest, 1970), lignin (AOAC, 1990; method number 973.18), and gross energy (GE) using a bomb calorimeter (Parr Instrument Co., Moline, IL). Urine was analyzed for N and energy as described above.

Data were analyzed according to a randomized block design using mixed model with the fixed effects of

treatment (two degrees of freedom, df), the random effects of blocks (4 df), and the random residual error using the MIXED procedure of SAS (Statistical Analysis System, version 9.4). When significant, the effect of level of feed restriction was decomposed into two orthogonal polynomial contrasts (linear and quadratic). Because levels were not equally spaced, coefficients were generated using the IML procedure of SAS (Statistical Analysis System, version 9.4). Results were considered statistically significant at P<0.05 and tendency at 0.05<P≤0.10.

Trial 2 comprised a single period of 26 days. Days 1 to 21 were used to adapt the animal to the diet. Goats were distributed into five groups (blocks), according to BW and body condition score, with three animals per group. Within each group, goats were allocated to one of the three F:C diets (Table 1): 75:25, 46:54, and 25:75. Forage consisted of dehydrated corn (*Zea mays*) plants and concentrate contained cracked corn grain, soybean (*Glycine max*) meal, molasses, soybean oil, limestone, and a mineral supplement. Feed was weighed and offered twice daily, at 08.00 and 16.00 h.

Procedures of the metabolism assay, measurement of methane, laboratory analyses, and energy balance calculations were similar to those previously described in Trial 1.

Data were analyzed according to a randomized block design using mixed model with the fixed effects of treatments as fixed effect (2 df) and the random effects of blocks (4 df) and the random residual error using the MIXED procedure of SAS (Statistical Analysis System, version 9.4). The effect of F:C ratio in the diet was decomposed into two orthogonal polynomial contrasts (linear and quadratic). Because levels were not equally spaced, coefficients were generated using the IML procedure of SAS (Statistical Analysis System, version 9.4). Results were considered statistically significant at P<0.05 and tendency at 0.05<P≤0.10.

Results

Body weight of goats was similar among levels of feed intake (Table 2). As expected, DM, nutrients, and energy intake decreased linearly with increasing levels of feed restriction. Despite the differences in intake, apparent total tract digestibility of DM, organic matter (OM), crude protein (CP), and NDF did not differ among treatments.

In this study, the animals were fed near ME requirements for maintenance, with level of intake (L) ranging from 1.35 to 0.74 (Table 2). Methane emission (g d⁻¹) decreased linearly as the intake level decreased. However, energy loss in methane proportional to OM intake was similar

among levels of feed restriction; consequently, dietary metabolizability (q) did not differ among treatments, either.

Methane emission (g d^{-1}) as a function of F:C ratio revealed a quadratic response (Table 3), showing the highest values when animals were fed the 46:54 F:C ratio diet (18.2 g d^{-1}), suggesting that the decrease in absolute methane production occurred when the level of inclusion of concentrate in the diet surpassed 50%.

The increased proportion of concentrate in the diet was accompanied by an increase in apparent DM digestibility (Table 3) because of the higher digestible OM content of the high-grain diet used. The increasing F:C level of the diet did not affect percentage of ingested GE converted to methane, which averaged $5.8 \pm 0.6\%$ of GE intake. Other energy-related variables (digestible energy (DE), metabolizable energy (ME), ME/DE, and metabolizability) increased by approximately 15% when the forage proportion in diet decreased from 75 to 25% in high-grain diets.

Discussion

As expected, DM, nutrients, and energy intakes decreased linearly with increasing levels of feed restriction. Our results did not indicate the negative relationship between intake and digestibility reported previously

(Colucci et al., 1990), presumably because the negative relationship between these variables has been established in experiments conducted at levels of intake above maintenance (Doreau et al., 2003). Conversely, the lack of intake level effects on digestibility was also observed in other studies (Puchala et al., 2005; Tovar-Luna et al., 2011), in goats fed near maintenance. In this study, the animals were fed near ME requirements for maintenance, with the level of intake (L) ranging from 1.35 to 0.74 (Table 2), and with a highly digestible diet, which may have contributed to maintaining the ruminal fermentation pattern even in conditions of low intake, possibly suggesting no effect of intake level on the passage rate of digesta throughout the gastrointestinal tract.

This positive relationship of DMI with ruminal CH_4 emission is in accordance with previous reports (Boadi et al., 2002; Molano and Clark, 2008). However, energy loss in methane proportional to OM intake was similar among levels of feed restriction; consequently, dietary metabolizability (q) did not differ among treatments either. A previous study pointed out that, during periods of feed restriction, animals experience metabolic and ruminal environmental changes to offset the low feed allowance (Doreau et al., 2003), which may impact their performance. Nonetheless, the high-quality diet offered to animals in this

Table 2 - Intake, digestibility, methane emission, and energy balance of goats subjected to feed restriction

Variable	Level of feed restriction ¹			P-value	SEM	Contrast ²	
	0	15	40			L	Q
Body weight (kg)	33.2	34.6	34.6	0.20	2.6	-	-
Intake (g d^{-1})							
Dry matter	775	653	453	<0.001	99	<0.001	-
Organic matter	716	608	423	0.001	93	<0.001	-
Crude protein	155	127	88	0.001	20	<0.001	-
Neutral detergent fiber	235	218	150	0.01	33	0.004	-
Gross energy (MJ d^{-1})	13.2	11.1	7.7	<0.001	0.89	<0.001	-
Apparent digestibility coefficient (g g^{-1})							
Dry matter	0.78	0.73	0.77	0.16	0.019	-	-
Organic matter	0.77	0.72	0.75	0.17	0.025	-	-
Crude protein	0.80	0.76	0.77	0.32	0.023	-	-
Neutral detergent fiber	0.59	0.54	0.62	0.21	0.031	-	-
Gross energy	0.76	0.71	0.76	0.15	0.022	-	-
Energy balance							
ME intake (MJ d^{-1})	8.6	6.7	4.8	0.002	1.27	<0.001	-
Methane (g d^{-1})	18.1	15.0	11.5	0.002	1.58	<0.001	-
Methane (MJ d^{-1})	1.00	0.82	0.63	0.002	0.087	<0.001	-
Methane (g kg^{-1} OM)	27.3	25.3	28.1	0.41	2.47	-	-
Urinary excretion (MJ d^{-1})	0.45	0.42	0.34	0.01	0.050	0.004	-
Fecal excretion (MJ d^{-1})	3.13	3.13	1.88	0.02	0.41	0.008	-
q	0.64	0.59	0.62	0.24	0.0303	-	-
L	1.35	1.01	0.74	0.003	0.18	0.001	-
ME/DE	0.84	0.83	0.82	0.29	0.019	-	-

BW - body weight; OM - organic matter; ME - metabolizable energy; DE - digestible energy; q - metabolizability; L - ratio between ME intake and ME required for maintenance ($462 \text{ kJ/kg}^{0.75} \text{ BW}$; NRC, 2007); ME/DE - ratio between ME and digestible energy of diet; SEM - standard error of the mean.

¹ 0 - no restriction, fed *ad libitum*; 15 - 15% feed restriction (equivalent to 85% of *ad libitum* intake); 40 - 40% feed restriction (equivalent to 40% of *ad libitum* intake).

² L - linear effect; Q - quadratic effect.

study, which contained a lower proportion of structural carbohydrates, did not induce enough changes in the rumen environment to affect CH₄ yield per unit of OMI. The lack of changes in diet digestibility supports this hypothesis.

The curvilinear response of methane emission (g d⁻¹) is consistent with previous studies (Lovett et al., 2003; Sauvant and Giger-Reverdin, 2007; Pedreira et al., 2013). As a matter of fact, the increment in concentrate levels from 25% to 50% replaces structural carbohydrates (cellulose, hemicellulose) from forages with non-structural carbohydrates (starch and sugars) present in most high-energy concentrates, inducing an increase in feed intake, rates of ruminal fermentation and feed turnover, with a consequent raise in CH₄ production (Martin et al., 2010). Above 50% concentrate, modifications of rumen physico-chemical conditions and microbial populations lead to a shift in volatile fatty acid (VFA) production from acetate towards propionate (Pedreira et al., 2013; Ribeiro et al., 2015). This results in low acetate:propionate ratio concomitant with a reduced ruminal pH and protozoal number, which have been suggested to inhibit growth and/or activity of methanogens (Hegarty, 1999; Aguerre et al., 2011) and of cellulolytic bacteria (Brossard et al., 2004).

In fact, Ribeiro et al. (2015) observed that the replacement of roughage with concentrate decreases the

rumen pH, since grains are usually more digestible than forages, resulting in accumulation of VFA. This substantial reduction of ruminal pH resulted in a reduction of fibrolytic bacteria, including *F. succinogenes*, *R. albus*, and *R. flavefaciens*, and an increase in bacteria that are consumers and producers of lactic acid. In this study, the increased consumption of non-fiber carbohydrates, as a consequence of increasing the proportion of concentrate in diet, might contribute to optimizing rumen fermentation, increasing propionogenesis (Doreau et al., 2011). This increase in the concentration of propionic acid in the rumen may lead to a decrease in production of enteric methane as the propionate in the procedure uses hydrogen, thereby not sparing hydrogen required for the production of methane for the methanogenic bacteria.

Islam et al. (2000) found that the inclusion of corn from 0 to 60% in the dietary DM increased DM apparent digestibility of goats fed alfalfa pellets. On the other hand, NDF digestibility did not change (Table 3), probably due to the nature of the forage used in this experiment, which was dehydrated corn plant chopped through a 4 mm screen. According to Hook et al. (2010), grinding forage increases the rate of digestion and passage through the gastrointestinal tract, thus limiting the time available for fermentation within the rumen. Indeed, increased passage rates can

Table 3 - Intake, digestibility, methane emission, and energy balance of goats subjected to different forage:concentrate ratios in the diet

Variable	Forage:concentrate ratio			P-value	SEM	Contrast ¹	
	25:75	46:54	75:25			L	Q
Body weight (kg)	38.8	37.7	39.1	0.57	3.2	-	-
Intake (g d ⁻¹)							
Dry matter	1000	1003	753	0.24	126	-	-
Organic matter	941	942	697	0.22	118	-	-
Crude protein	198	202	142	0.16	25	-	-
Neutral detergent fiber	251	322	339	0.16	35	0.083	-
Gross energy (MJ d ⁻¹)	17.0	17.2	12.6	0.20	2.1	-	-
Apparent digestibility coefficient (g g ⁻¹)							
Dry matter	0.83	0.79	0.74	0.02	0.018	0.01	-
Organic matter	0.82	0.76	0.73	0.02	0.019	0.01	-
Crude protein	0.82	0.76	0.76	0.15	0.021	-	-
Neutral detergent fiber	0.61	0.56	0.67	0.12	0.033	-	-
Gross energy	0.83	0.76	0.74	0.01	0.018	0.006	-
Energy balance							
ME intake (MJ d ⁻¹)	12.9	11.6	7.8	0.12	1.73	0.052	-
Methane (g d ⁻¹)	15.4	18.2	15.6	0.03	1.25	-	0.01
Methane (MJ d ⁻¹)	0.85	1.00	0.86	0.03	0.071	-	0.01
Methane (g kg ⁻¹ OM)	17.6	19.9	22.9	0.28	2.59	-	-
Urinary excretion (MJ d ⁻¹)	0.48	0.52	0.50	0.88	0.060	-	-
Fecal excretion (MJ d ⁻¹)	2.84	4.13	3.27	0.13	0.46	-	0.06
q	0.74	0.67	0.63	0.009	0.022	0.004	-
L	1.78	1.69	1.12	0.096	0.21	0.042	-
ME/DE	0.90	0.88	0.85	0.074	0.014	0.027	-

BW - body weight; OM - organic matter; ME - metabolizable energy; DE - digestible energy; q - metabolizability; L - ratio between ME intake and ME required for maintenance (462 kJ/kg^{0.75} BW; NRC, 2007); ME/DE - ratio between ME and digestible energy of diet; SEM - standard error of the mean.

¹ L - linear effect; Q - quadratic effect.

shift fermentation to the hind gut, possibly offsetting any reduction of ruminal digestibility, resulting in a lack of difference in total tract digestibility between high-forage and high-concentrate diets.

Our findings were similar to those reported by Beauchemin and McGinn (2006), who did not find differences in methane conversion rate (% to GE) of steers fed high-grain (70% grain on DM basis) and high-forage (70% forage on DM basis) diets based on barley silage and corn grain. Ruminal methane depends on the quality of the diet ingested (Shibata and Terada, 2010; Hristov et al., 2013), and represents losses ranging from 5 to 7% of dietary GE and methane emissions ranging from 10 to 16 kg CH₄ per year in sheep and goats (Hristov et al., 2013). In this study, increasing forage concentration in the diet did not alter GE intake, probably as a result of the ability of goats to select high-quality feed (Silanikove, 2000). Consequently, similar intakes of GE associated with high digestible fiber content of diets, irrespective of the forage level, resulted in a lack of variation in percentage of ingested gross energy intake converted to methane. On the other hand, other energetic variables (DE, ME, ME/DE, and metabolizability) increased by approximately 15% when the forage proportion decreased from 75 to 25% in high-grain diets, in which the greater proportion of rapidly fermentable carbohydrates provides the best energy use possible.

Conclusions

Methane emission is positively affected by the level of feed restriction. Moreover, the relationship between concentrate proportion and CH₄ production is curvilinear, with absolute methane production decreasing when the level of concentrate inclusion in the diet exceeds 50%. The results presented herein may be relevant for the ongoing and future efforts towards completion of IPCC inventory regarding the contribution of goats to the greenhouse gas effects on the planet.

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